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The effect of polyvinylpyrrolidone nanowires on the metabolic activity of Lactobacillus acidophilus

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Abstract. The effectiveness of the use of polyvinylpyrrolidone (PVP) nanowires for increasing the metabolic activity of probiotic cultures of *Lactobacillus acidophilus* has been shown. PVP nanowires are obtained by electrospinning in two versions: PVPI based on a solution of high molecular weight polyvinylpyrrolidone and distilled water; PVPII based on a solution of two water zinc acetate in distilled water and a solution of high molecular weight PVP in ethanol. In the presence of PVP, there was an increase in viable lactobacillus cells up to 7.4×10^8 CFU/ml and accumulation of DL-lactic acid during acidification of the test solution to 3.10 and an increase in Eh to + 360.5 mV. PVPI is a more preferred carrier for lactobacilli than PVPII with ZnO. The zinc ions contained in the PVP matrix are capable of causing oxidative stress, followed by the formation of free radicals that damage membrane lipids, proteins and DNA of the prokaryotic cell. The results are aimed at developing new forms of immobilized drugs that can be used as alternative probiotic agents.

1.Introduction

Poly-(N-vinylpyrrolidone) refers to synthetic polymers-gamma-vinyl lactam N-aminobutyric acid, obtained by homopolymerization of an aqueous solution of N-vinylpyrrolidone at a temperature of 50-80 °C under the action of 0.05-2.5% H_2O_2 in the presence of NH_3. Due to the complex of physical, chemical and biological properties, polyvinylpyrrolidone (PVP) is a unique compound, has biocompatibility and the ability to interact with cell membranes, non-toxicity, chemical stability and solubility in water and organic solvents [1]. According to the degree of impact on the human body, PVP belongs to the 4th hazard class or low hazardous substances, has an affinity for complex hydrophobic and hydrophilic substances, and is capable of forming complexes with transition metal ions, drugs and toxic compounds of different origin. The listed PVP properties have found application

in medical technologies, in optics and electrical engineering, on its basis created highly permeable composite membranes with a selective layer, adhesives, cetamines, various coatings and fibers [2; 3]. The pharmaceutical industry uses the ability of PVP to bind the functional components of a drug until the drug reaches the stomach, and then dissolve quickly to release the drug. Using studies in this direction, it has been proved that PVP can also have the opposite effect if it is used as a coating and delayed the release of a drug in order to have a prolonged effect and eliminate multiple dosages. Thus, the use of PVP as a coating for venlafaxine-montmorillonite antidepressants is described in order to slow the release of venlafaxine [4].

PVP nanofibres obtained by electrospinning are used in tissue engineering as a biocompatible extracellular matrix [5]; PVP and emodin membranes, as well as PVP and alginate hydrogel nanowires with silver nanoparticles in the form of AgNPs are successfully used for drug delivery and accelerated wound healing [6]. There is evidence of magnetic nanoparticles coated with silica and embedded in PVP fibers obtained by electrospinning. In the experiment, modified PVP nanoparticles showed unique fluorescent and magnetic properties [7]. There are data on the development of films based on chitosan and PVP doped with AgNPs with prolonged bactericidal activity, which is promising for solving the problems of device-associated infections [8]. Studies on the encapsulation of DNA molecules by PVP nanoparticles were performed [9]. When DNA was immobilized with PVP, it was found that 80% transfection efficiency is observed in vitro, which makes PVP an effective carrier of functional molecules for therapeutic delivery [9].

The purpose of the work performed is related to the study of the biological effect of PVP nanowires on *Lactobacillus acidophilus* microorganisms with a pronounced probiotic potential. Compared with other lactobacilli, *L. acidophilus* are the most recommended microbial cultures for use as probiotics. They are involved in the restoration of microintestinal balance [10; 11], modify the activity of bacterial enzymes, affect the permeability of the intestinal mucosa and the regulation of the immune system [12; 13]. The metabolic products of probiotic cultures exhibit antibiotic properties and inhibit the vital activity of pathogens of the genus *Klebsiella*, *Enterobacter, Pseudomonas, Salmonella, Serratia, Bacteroides*, and others [12]; Probiotics control the level of glucuronidase, β-glucuronidase, nitroreductase and azoreductase in the intestine. These enzymes catalyze the conversion of procarcinogens into carcinogens, such as nitrosamine and secondary bile acids [13; 14]. Low levels of these enzymes significantly reduce the risk of cancer in the colon. It has been suggested that lactobacilli immobilized by modified PVP nanofibres are capable of increasing their metabolic activity and long-lasting viability with prolonged probiotic action.

2. Method

The effect of polyvinylpyrrolidone nanowires was tested on cells of the *L. acidophilus* strain Ep. n. v. 317/402 in the composition of the commercial preparation «Narine», series 500051. The preparation contains lyophilisate *L. acidophilus* in the amount of 1×10^9 CFU / g, corn starch, sucrose and magnesium stearate (Mg $(C_{18}H_{35}O_2)_2$. Lactobacillus cultures were grown under experimental and control conditions on agar media of the recommended composition [15]. In the experiment, 0.075 g of PVPI (option 1) and 0.085 g of PVPII with ZnO (option 2) were added to the nutrient medium. In the control, lactobacilli were cultured without adding PVP. solution based on high molecular weight of polyvinylpyrrolidone (Mr = 1.3×10^6 g / mol) and distilled water at a rate of 0.13 g / ml; PVPII - when mixing a solution of two aqueous zinc acetate Zn $(CH_3COO)_2 \times 2H_2O$ in distilled water and a solution of high-molecular PVP (Mr = 1.3×10^6 g / mol) in ethanol. A medical syringe (needle diameter 0.7 mm) was used in an electrospinning unit. The PVPI and PVPII solutions were supplied from the syringe with a NE-300 syringe pump at a rate of 0, 5 ml / hour An electric field with a strength of 1.8 kV / cm was created between the needle and the metal substrate with the help of a high-voltage source «AHVS -30/5» (adjustable high voltage source). The diameter of the obtained filaments 200 - 300 nm. The photo of the obtained PVP nanowires is presented in Figure 1.

Figure 1. Photo of PVP nanowires, magnification 80 ×.

The metabolic activity of immobilized *L. acidophilus* strains was assessed by enzyme activity and the formation of DL-lactic acid. The presence of DL-lactic acid was determined by the results of a qualitative reaction with H_2SO_4, $CuSO_4 \times 5H_2O$ and thiophene solution. Additionally, we analyzed the pH and redox potential (Eh) of the medium, which are technological parameters and control the direction of biochemical processes; The experiment also studied the morphology and cultural properties of bacteria. The morphology of the lactobacillus colonies was analyzed using a «MOTIC DM-BA-30» biological microscope, the morphology and cell linear dimensions – using SEM microscopy using a «HITACHI SU1510» microscope with an energy dispersive attachment. To do this, microbial cells were applied to the surface of a microscope slide, dried, fixed in ethyl alcohol, followed by applying a layer of gold by ion sputtering using a magnetron sputtering system and mounted on a microscope stage. *Lactobacillus* microscopy was performed under a high vacuum condition. The viability of lactobacilli in the experiment and control was taken into account within 30 days of the experiment according to the results of planting the corresponding tenfold dilutions of the suspensions studied on agar media followed by counting the colony forming units after the incubation time. Statistical processing of the data was performed according to the methods used in microbiological studies [16].

3. Results and Discussion

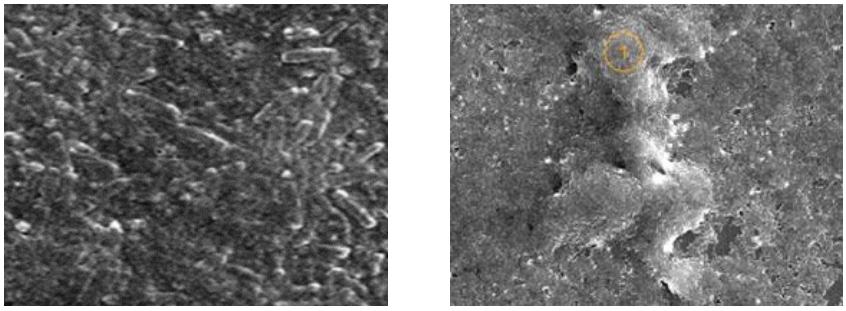
In the experiment with PVPI and PVPII, lactobacilli actively adhered to the surface of the carrier, which led to an increase in the number of viable cells from $3{,}1 \times 10^3$ CFU / ml (PVPII, 24 h) to $7{,}4 \times 10^8$ CFU / ml (PVPI, 30 days) . In the control, the number of actively growing bacteria did not exceed $3{,}4 \times 10^5$ CFU / ml, and by the end of the experiment it decreased to $2{,}5 \times 10^5$ CFU / ml (Table 1). Based on the above data, it should be noted that the nutrient medium with 0.075 g of PVPI was preferable for activating the growth and reproduction of microbial cells than the medium with the addition of 0.085 g of PVPII with ZnO, in all cases an increase in the share of viable microorganisms from 8.8% (24 h) up to 44.6% (30 days).

Table 1. The number of viable cells (CFU / ml-1) of *L. acidophilus* in experiment and control (X ± I95)

The period of immobilization	PVPI	PVPII	Control
24 hours	$(3{,}4 \pm 0{,}4) \times 10^4$	$(3{,}1 \pm 0{,}4) \times 10^3$	$(2{,}1 \pm 0{,}7) \times 10^4$
10 days	$(4{,}3 \pm 0{,}6) \times 10^6$	$(3{,}7 \pm 0{,}4) \times 10^4$	$(2{,}8 \pm 0{,}7) \times 10^6$
20 days	$(5{,}9 \pm 0{,}5) \times 10^8$	$(4{,}7 \pm 0{,}6) \times 10^6$	$(3{,}4 \pm 0{,}6) \times 10^5$

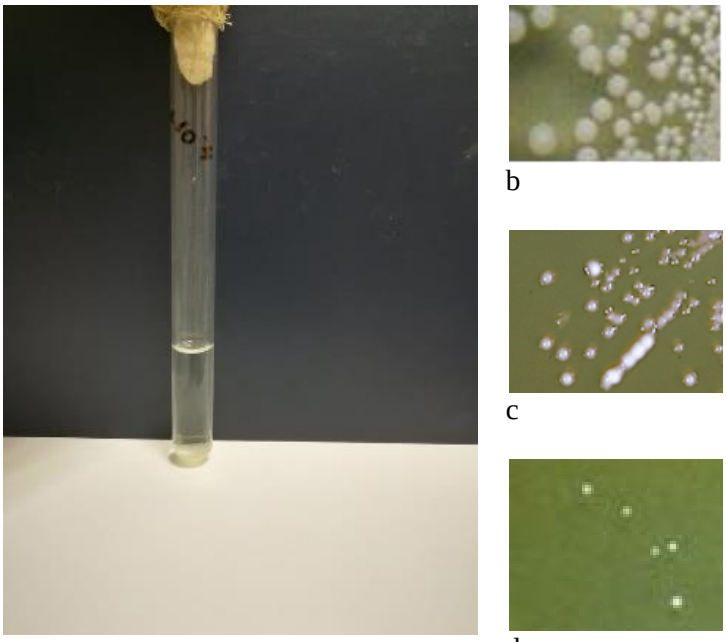
30 days	$(7,4\pm0,6) \times 10^8$	$(4,1\pm0,6) \times 10^7$	$(2,5\pm0,7) \times 10^5$

As a result of SEM microscopy, in the experiment and control, the presence of large straight rods with rounded ends, located singly or in chains of 2-3 cells (Fig. 2a) and large globular structures of exopolysaccharide nature (Fig. 2b), was established as a result of an increase in metabolic activity of immobilized cells. Lactobacilli are immersed in the semi-liquid matrix of exopolysaccharide and are practically not visible on electronic photographs. Despite the morphological uniformity of bacteria grown both in the presence of PVP and without it, the culture characteristics (size of colonies) of the control and experimental variants of lactobacilli on agar nutrient media were significantly different. The maximum diameter of colonies (0.29 ± 0.06 cm) reached the bacteria in the presence of PVPI (Fig. 3b), in the presence of PVPII, the diameter of the colonies was 0.34 ± 0.06 cm (Fig. 3c), and in the control it was not exceeded 0.20 ± 0.05 cm (Fig. 3d).



a (×3000) b (×1000)

Figure 2. Electronic photographs of immobilized PVP *L. acidophilus* (made using a «HITACHI SU1510» scanning electron microscope, gold plating)



a b c d

Figure 3. The appearance of the tubes with the appropriate dilution of *L. acidophilus* (10-4) (a); photographs of the culture growth of *L. acidophilus* on agar media in the experiment with PVPI (b), PVPII (c) and in the control (d)

The effect that stimulates the metabolic activity of bacteria caused by the presence of polyvinylpyrrolidone is confirmed by the results of studying the dynamics of pH and Eh in experiment and control (Table 2 and 3). For 30 days of the experiment, the pH of the culture medium due to the accumulation of lactate decreased to 3.10 (PVPI) and to 3.80 (PVP II). In the control, the pH values were only 4.50. Apparently, such acidity was found to be a threshold for native lactobacilli, which, with the depletion of the nutrient substrate and the accumulation of extracellular toxic metabolites, caused the inhibition of the growth of microorganisms and a decrease in their viability. The values of Eh in the experimental conditions steadily increased from +102 mV to +360 mV (for PVPI) and from +90 mV to +342 mV (for PVPII). In the control, an increase in Eh was observed only during the 20 days of the experiment (from +72 mV to +203 mV), and by 30 days – Eh decreased to +169 mV (Table 2).

Table 2. Dynamics of pH and Eh of the culture medium in the experiment and control

Period of immobilization	PVPI	PVPII	Control
24 hours	4,92*	5,20	6,10
	102,7**	90,4	72,8
10 days	3,70	4,62	5,89
	258,8	210,7	107,1
20 days	3,24	4,10	4,43
	311,4	260,1	203,2
30 days	3,10	3,80	4,50
	360,5	342,4	169,7

Note: * - pH values, ** - Eh values

The presence of DL-lactic acid was detected in all variants of the experiment and in the control (Table 3).

Table 3. Titratable acidity of the culture medium in the experiment and control

Period of immobilization	PVPI	PVPII	Control
24 hours	80 DL MK +	70 DL MK +	50 DL MK +
10 days	110 DL MK +	90 DL MK +	100 DL MK +
20 days	140 DL MK +	180 DL MK +	130 DL MK +
30 days	310 DL MK +	220 DL MK +	130 DL MK +

However, the maximum increase in titrated acidity by 30 days of the experiment was recorded for PVPI, and the minimum - for control.

4. Conclusions

According to the results of studies of the biological effect of PVP nanowires on *L. acidophilus*, we can state the proven positive effect of polyvinylpyrrolidone nanowires on the metabolic activity of probiotic cultures. Immobilized PVP cells of lactobacilli are characterized by rapid expression of

genes controlling the catabolism of lactose. In the presence of PVP, cells maintain a long-term viability level compared with lactobacilli growing in liquid suspension cultures. Probably, the growth of microorganisms in the presence of PVP nanowires determines the degree of cell aggregation, having an indirect effect on the synthesis of secondary metabolites. It should be noted that zinc ions contained in the PVP matrix can cause oxidative stress with the subsequent formation of free radicals, damaging lipid membranes, proteins and DNA of the prokaryotic cell. This, in turn, regulates a cascade of mechanisms that accelerate metabolic processes associated, in this case, with the splitting of lactose to lactic acid and carbon dioxide. Subsequent acidification of the culture mixture (to pH 3.1), apparently, triggers the expression of genes that ensure the formation of biofilm formation in lactobacilli cells immobilized on PVP. The results will be aimed at developing new forms of immobilized drugs that can be used as alternative probiotic agents. The work was performed within the framework of the state assignment of the Ministry of Education and Science of Russia № 16.5857.2017 / 8.9 and the implementation of the Development Program of the support university of FSBEI HE «Petrozavodsk State University» for the period 2017-2021.

References

[1] Teodorescu M and Bercea M 2015 Poly(vinylpyrrolidone) – a versatile polymer for biomedicaland beyond medical applications *Polymer-Plastics Technology and Engineering* 54 pp 923–943
[2] Osadchenko S et al 2013 New Composite Membranes with a Selective Layer Based on the Polyaniline-Poly- (N-Vinyl Pyrrolidone) System *Bulletin of the Nizhny Novgorod University* I Lobachevsky 2 (1) pp 79-85
[3] Zhi X *et al* 2013 The immunotoxicity of graphene oxides and the effect of PVP-coating *Biomaterials* 34 pp 5254–5261
[4] Patel H A *et al* 2011 Sustained release of venlafaxine from venlafaxine–montmorillonite polyvinylpyrrolidone composites *Appl. Clay Sci.* 51 pp 126–130
[5] Wang M *et al* 2014 Preparation of PVDF/PVP core–shell nanofibers mats via homogeneous electrospinning *Polymer (Guildf)* 55 (9) pp 2188–2196
[6] Dai X Y *et al* 2012 Electrospun emodin polyvinylpyrrolidone blended nanofibrous membrane: a novel medicated biomaterial for drug delivery and accelerated wound healing *J. Mater. Sci. Mater. Med.* 23 pp 2709–2716
[7] Chen M *et al* 2012 *Magnetic electrospun fluorescent polyvinylpyrrolidone nanocomposite fibers Polymer (Guildf)* 53 pp 4501–4511
[8] Wang B L *et al* 2012 Fast and long-acting antibacterial properties of chitosan Ag/polyvinylpyrrolidone nanocomposite films *Carbohydr. Polym*. 90 pp 8–15
[9] Saxena A *et al* 2006 Ultra-low sized cross-linked polyvinylpyrrolidone nanoparticles as non viral vectors for in vivo gene delivery *Biomaterials* 27 pp 5596–5602
[10] Betoret N *et al* 2003 Development of probiotic-enriched dried fruits by vacuum impregnation *J. of Food Engineering* 56 pp 273-277
[11] Sullivan A and Nord C E Probiotics and gastrointestinal diseases *J. of Internal Medicine* 257 pp 78-92
[12] Krasaekoopt W *et al* 2003 Evaluation of encapsulation techniques of probiotics for yoghurt *International Dairy Journal* 13 pp 3-13
[13] Adams M R and Moss M O 2000 Food Microbiology *Second Edition, The Royal Society of Biochemistry* (Cambridge, UK) pp 318-323
[14] Gorbach S L 1997 Health benefits of probiotics *IFT Annual Meeting, Orlando, FL, Abstract* pp 73-81
[15] Likhacheva A Yu et al 1992 The current state of the issue of the taxonomy of bacteria of the genus Lactobacillus Journal of Microbiology 9-10 pp 74-78
[16] Ashmarin I P and Vorobiev A A 1962 Statistical methods in microbiological research *L.: Medgiz* p 280